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(FILE 'HOME' ENTERED AT 15:15:15 ON 18 MAY 2004)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 15:15:37 ON 18 MAY 2004

L1 1676 S HIV? (P) (SUBTYPE?) (P) (AMPLIF? OR PRIMER? OR PCR)
L2 421 S L1 (P) (SPECIFIC OR DISCRIMINA? OR DISTINGUISH)
L3 123 DUP REM L2 (298 DUPLICATES REMOVED)

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L Number	Hits	Search Text	DB	Time stamp
1	504	hIV same (subtypes or isolates) same env	USPAT; US-PGPUB; DERWENT	2004/05/18 15:08
2	76	(hIV same (subtypes or isolates) same env) same (PCR or amplif$ or primer$)	USPAT; US-PGPUB; DERWENT	2004/05/18 15:09

DOCUMENT-IDENTIFIER: US 20030148266 A1

TITLE: Reference clones and sequences for non-subtype B isolates of human immunodeficiency virus type 1

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Detail Description Paragraph - DETX (109):

[0160] All newly derived HIV-1 genome sequences were aligned with previously reported (45) full length representatives of HIV-1 subtype A (U445), B (LAI, RF, OYI, MN, SF2), C (C2220), D (ELI, NDK, Z2Z6), and "E" (90CF402.1, 93TH253.3, CM240) as well as SIVcpzGAB as an outgroup using the CLUSTAL W (67) profile alignment option (the alignment includes the untranslated leader sequence, gag, pol, vif, vpr, tat, rev, vpu, env, nef and available 3' LTR sequences). Sequences that needed to be excluded from any particular analysis were removed only after gap-tossing was performed on the complete alignment containing all sequences. This ensured that all positions were comparable in different runs with different sequences.

Detail Description Paragraph - DETX (123):

[0173] Of the geographically diverse HIV-1 isolates described herein, five had previously been classified as members of (group M) subtypes A (92RW009), F (92BR020, 92BR029), and G (92NG003, 92NG083) on the basis of env (17,19) and/or gag sequences (1). One (90CF056) was chosen because it originated from a major epicenter of the African AIDS epidemic. In addition, 90CF056 did not fall into any known subtype at the time of its first genetic characterization (43).

Isolates from Zambia (96ZM651 and 96ZM75 1) and India (94IN476) were chosen because of the known subtype C prevalence in those countries. The two isolates from Cyprus (94CY017 and 94CY032) were selected because of the extensive diversity of HIV-1 in the drug user population (29). Table 1 summarizes available demographic and clinical information, as well as biological data concerning the isolate phenotype (SI/NSI). Only viruses grown in normal donor PBMCs were selected for analysis.

Detail Description Paragraph - DETX (126):

[0176] All eleven HIV-1 genomes were sequenced in their entirety using either shotgun sequencing or primer walking approaches. The long PCR derived clones ranged in size from 8,952 to 8,999 base pairs, and spanned the genome from the primer binding site to the R/U5 junction of the 3' LTR. Inspection of potential coding regions revealed that all clones contained the expected reading frames for gag, pol, vif, vpr, tat, rev, vpu, env and nef. In addition, all major regulatory sequences, including promotor and enhancer elements in the LTR, the packaging signal, splice sites, etc., appeared to be intact. None of the genomes had major deletions or rearrangements, although inspection of the deduced protein sequences identified inactivating mutations in seven of the eleven clones (Table 2). However, most of these were limited to point mutations in single genes and were thus amenable to repair. Only two genomes (92NG003.1 and 92NG083.2) contained stop codons, small deletions and frameshift mutations in several genes, rendering them multiply defective. Importantly, no inactivating mutations were identified in 93BR020.1 (subtype F), 90CF056.1 (subtype H), and 96ZM651.8 (subtype C), suggesting that these

clones encoded biologically active genomes (Table 2). Nucleic acids containing repaired coding sequences, as well as the polypeptides encoded by the repaired coding sequences, are also considered to be a part of the invention.

Detail Description Paragraph - DETX (156):

[0200] Full length reference clones and sequences are currently available for eight HIV-1 group M subtypes (A-H), but none have been reported for subtypes I and J, which have only been identified in a handful of individuals. Phylogenetic information for subtype I, in particular, is limited since only a very small env gene fragment (400 bp in the C2-V3 region) obtained from only two individuals (a heterosexual couple of intravenous drug users from Cyprus) has been analyzed. To characterize subtype I in greater detail, long range PCR was employed to clone a full length provirus (94CY032.3) from a short-term cultured isolate (94CY032) established from one of the two individuals originally reported to be infected with this subtype.

Detail Description Paragraph - DETX (158):

[0202] The complete sequence of 94CY032.3 was determined using the primer walking approach [GenBank accession numbers: AF049337 (genome) and AF049338 (LTR)]. Examination of potential coding regions revealed the expected reading frames for gag, pol, vif, vpr, tat, rev, vpu, env and nef (FIGS. 13A-13Z). None of the genes contained major deletions, insertions or rearrangements. However, both env and vif genes contained single in-frame stop codons (FIGS. 13A-13Z). There was also a frameshift at position 5199 (single base pair insertion) which altered the C-terminus (last six amino acid residues) of the Vpr protein. All other protein domains of known function

as well as major
regulatory sequences, including the primer binding site,
the packaging signal
and major splice sites, appeared to be intact. Similarly,
the number, position
and consensus sequences of promoter and enhancer elements
in the 94CY032.3 LTR
were indistinguishable from those of most other HIV-1
strains, except for the
presence of an unusual TATA sequence (TAAAA), thus far only
found in "subtype
E" (A/E) viruses from Thailand and the Central African
Republic (7, 18).